REMARKS

Applicants have amended their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended claim 1 to incorporate therein the subject matter of claim 8, and to further recite that each of the two or more types of probes comprises several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases. In light of amendments to claim 1, claim 8 has been cancelled without prejudice or disclaimer and, dependency of claim 9 has been amended; and claim 14 has been amended in light of recitation of two or more probes in claim 1.

Moreover, Applicants are adding new claims 15 and 16 to the application. Claims 15 and 16, each dependent on claim 1, respectively recites that the two or more types of probes respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other; and recites that a number of module sequences constituting each probe is in a range of 5-8.

In connection with amendments to previously considered claims, and in connection with the new claims, note Item 5 on pages 14-18 of Applicants' specification, especially together with Figs. 4 and 5 of Applicants' disclosure.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims as formerly in the application, that is, the teachings of U.S. Patent No. 6,110,681 to Ovyn, et al., and the articles by Whitcombe, et al., "A homogenous fluorescence assay for PCR amplicons: Its application to real-time, single-tube genotyping", in Clinical Chemistry, 44:5 (1998),

pages 918-923; Rizzo, et al., "Chimeric RNA-DNA molecular beacon assay for ribonuclease H activity", in Molecular and Cellular Probes (2002) 16, 277-283; Leone, et al., "Molecular beacon probes combined with amplification by NASBA enable homogenous, real-time detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155 (Leone '98), and Leone, et al., "Direct detection of potato leafroll virus in potato tubers by immunocapture and the isothermal nucleic acid amplification method NASBA", in Journal of Virological Methods 66 (1997), pages 19-27 (Leone '97), under the provisions of 35 USC 103.

It is respectfully that the references as applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having processing steps as in claim 1, including, inter alia, subjecting a gene to be analyzed to nucleic acid amplification using, inter alia, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene being further defined, and a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease, with two or more target genes being simultaneously detected in a single reaction vessel using two or more types of probes, and wherein each of the two or more types of probes comprise several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other and each probe being constituted by rearranging the order of the module sequences having identical terminal bases. See claim 1.

As discussed further infra, according to the present invention each of the two or more probes used in the present invention include several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by rearranging the order of the module sequences having identical terminal bases. By this specific structure of the probes, while the entire sequence of each probe is different from each other, these probes have substantially the same Tm (melting temperature) value, and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in a same reaction tube. Accordingly, accurate analysis can be made when these probes are used for quantitative analysis.

Furthermore, it is respectfully submitted that these references as applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and, in addition, wherein such at least two types of probes have substantially the same melting temperature. See claim 9.

In addition, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and, moreover, wherein the two or more types of probes respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other (see claim 15); and/or wherein a number of module sequences constituting each probe is in a range of 5-8 (see claim 16).

Moreover, it is respectfully submitted that the teachings of the references as applied by the Examiner would have neither disclosed nor would have suggested

such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having features as in the remaining dependent claims, including (but not limited to) wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction, as in claim 2; and/or wherein the nucleic acid amplification is conducted by steps as in claims 3 and 4; and/or wherein the nucleic acid amplification is conducted at a substantially single temperature (see claim 5), in particular, where such single temperature is between 37°C and 55°C (see claim 6); and/or wherein the RNA polymerase and the second base sequence are as set forth in claim 7; and/or wherein the probe is a DNA/RNA hybrid strand (see claim 14).

By use of the primer for introduction as in the present claims, which includes the first, second and third base sequences relatively located to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, the first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, together with the probe comprising a base sequence identical or complementary to the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The universal probe according to the present invention can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. Note, for example, the second paragraph on page 6 of Applicants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Furthermore, by using at least two types of probes as in the present claims, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by rearranging the order of the module sequence having additional terminal bases, the entire sequence of each probe is different from each other, but can have the same melting temperature value (note especially claim 9), and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in the same reaction tube, whereby accurate analysis can be made in a same reaction tube, of two or more target genes using two or more types of probes. Thus, accurate analysis can be made, when the probes are used for quantitative analysis.

Whitcombe, et al. discloses a method whereby a single TaqMan™ probe can be used for many polymerase chain reactions. The principal aim of the study reported in this article was to identify a means that the Amplification Refractory Mutation System (ARMS) could be exploited in a homogenous, high throughput and, in particular, an economical manner, and this article discloses that what was required was a way of using a single pair of allele-specific fluorescent probes for any bi-allelic polymorphism. For the fluorescent signal generation method, the authors of this article chose TaqMan™. The system used, as reported in the article, a 5'-exonuclease assay of amplicon annealed fluorogenic probes that operate in conjunction with the Amplification Refractory Mutation System, whereby relative changes in reporter fluorescent emission are monitored in real-time using an analytical thermal cycler, this system being called Three-STAR, and it is universal in that it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism. Note the first paragraph in the left-hand column on page 918, as well as the paragraph

bridging pages 918 and 919. Fig. 1 shows the scheme of the Three-STAR cycle. Note also the paragraph bridging the left- and right-hand columns on pages 921, describing that the authors have devised a way to make TaqMan™ generic inasmuch as that just one fluorogenic probe can be universally applied in any PCR reaction. See also Fig. 2 on page 921, showing single-tube genotyping.

It is respectfully submitted that Whitcombe, et al. discloses a probe non-specific to a target gene, and genotyping of 2 or more target genes in a single tube (see Fig. 2 and Table 1 on page 919). However, as recognized from the descriptions "homozygote" and "heterozygote" in the legend of Fig. 2, Whitcombe, et al. conducts typing of allele in a single sample. In contrast, two or more different target genes, e.g., derived from different samples, are detected in the present invention, using two or more probes having substantially the same Tm value. As described below, two or more probes shown in Table 1 of Whitcombe, et al. have different Tm values.

Thus, Tm values were calculated using the most accurate method, base-stacking Tm calculation (http://www.promega.com/biomath/default.htm). The FAM probe and TET probe, in Fig. 2 of Whitcombe, et al., are shown below, and respectively have melting temperatures of 65°C and 72°C:

FAM probe: CTGG CATC GGTA GGGT AAGG ATCG GTAT CG, 30mer, 65°C; and
TET probe: CGGT GGAC GTGA CGGT ACGA CGAG GCGA CG, 30mer, 72°C.

When Tm values are calculated using more simple methods, Basic Tm Calculations (66°C and 71°C) and salt-adjusted Tm calculations (61°C and 66°C) show that these two probes, i.e., FAM probe and TET probe, have different Tm values. Moreover, from the FAM probe and TET probe as set forth in the foregoing, it is clear that the two probes used in Whitcombe, et al. do not have the structure of the probes as recited in the present claims. Thus, it is respectfully submitted that

Whitcombe, et al. would have neither disclosed nor would have suggested various aspects of the present invention, including, inter alia, the probes used, and advantages due thereto.

Even taking into account the teachings of Ovyn, et al., it is respectfully submitted that the combined teachings of Whitcombe, et al. and of Ovyn, et al. as applied by the Examiner would have neither taught nor would have suggested the presently claimed subject matter, including, inter alia, the probes as recited in the present claims and as used in the presently claimed process.

Ovyn, et al. discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. See column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

Even assuming, arguendo, that it would have been obvious to one of ordinary skill in the art at the time the present invention was made to apply the process of Whitcombe, et al. into the method of NASBA amplification taught by Ovyn, et al., as contended by the Examiner, such combined teachings would have neither disclosed nor would have suggested the present invention, including the two or more types of probes used, as recited in the present claims, with two or more target genes being simultaneously detected in a single reaction vessel using these probes, whereby accurate quantitative analysis of the target genes can be achieved.

It is respectfully submitted that the additional secondary references applied by the Examiner would not have rectified the deficiencies of the combined teachings of Whitcombe, et al. and Ovyn, et al., such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

In connection with claim 14, the article by Rizzo, et al. discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labeled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA/DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusion set forth in the left-hand column on page 282, of this article.

Even assuming, arguendo, that the teachings of Rizzo, et al. were properly combinable with the teachings of Whitcombe, et al. and of Ovyn, et al., as applied by the Examiner, such combined teachings would have neither disclosed nor would have suggested the presently claimed method, including the two or more types of probes used, with two or more target genes being simultaneously detected in a single reaction vessel, and advantages thereof, as discussed previously, and/or such feature together with at least one of the probes being a DNA/RNA hybrid strand, as in claim 14.

Leone '98 discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacon technology to produce a homogenous assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the

headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

Leone '97 contains a description that the sense primers were entirely target specific, wherein the antisense primers consisted of a 3' terminal, target specific sequence and a 5' terminal T7 promoter sequence. See page 21, Section 2.2.

Whitcombe, et al. has been previously discussed.

Even assuming, arguendo, that the teachings of Whitcombe, et al. were properly combinable with the teachings of the two Leone articles, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes, the probes being defined as in the present claims, including, inter alia, both of the terminal bases of each module sequence of the respective probes are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases.

Furthermore, it is respectfully submitted that the combined teachings of Whitcombe, et al., the two Leone, et al. articles and Rizzo, et al., would have neither taught nor would have suggested the subject matter of claim 14.

The teachings of Whitcombe, et al. has been previously discussed, as has the teachings of each of Leone '98, Leone '97 and Rizzo, et al. Even assuming, arguendo, that the teachings of these references were properly combinable, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, detection of two or more target genes simultaneously in a single reaction vessel using two or more types of probes, with the two or more types of probes being further defined as in the claims, including,

inter alia, wherein both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, with advantages thereof as discussed previously, including, inter alia, wherein the melting temperatures of the probes are substantially the same (see claim 9).

In view of the foregoing comments and amendments, reconsideration and allowance of all claims presently pending in the above-identified application are respectfully requested.

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Respectfully submitted,

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